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SPERMATOPHORE FORMATION IN TWO INTERTIDAL CRABS *ALBUNEA SYMNISTA* AND *EMERITA ASIATICA* (DECAPODA: ANOMURA)

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ABSTRACT

Decapod crustaceans employ spermatophores in sperm transfer. Anomura spermatophores are generally pedunculate and structurally species-specific. Spermatophores of the sand crabs *Albunea symnista* and *Emerita asiatica* are macruran-type. The *A. symnista* spermatophore is non-pedunculate, and comprised of a highly convoluted tube with a firm membrane forming a cord-like mass. This spermatophoric ribbon is embedded in a gelatinous matrix. In *E. asiatica,* the spermatophores are dimorphic and pedunculate, and are attached by the peduncle-end in a row on strands of membrane. The whole spermatophoric ribbon is further embedded in a jelly-like matrix. The histology of the inner epithelial cells of the vas deferens contributing to the spermatophore mass is described. Histochemical observations on the various secretory materials and their transformation to various spermatophoric components are reported. Mucopolysaccharides are the main component. The occurrence of pedunculate spermatophores in *E. asiatica* and their absence in *A. symnista* may reflect an interesting phylogenetic interrelationship, but the epizoic attachment of spermatophores and the probable mode of dehiscence are discussed in relation to the adaptive strategies of the two crabs to the precarious intertidal zone.

INTRODUCTION

Arthropod spermatophores evolved to protect the sperm during their transfer to the females as the aquatic ancestors moved to dry land (Schaller, 1980). While most highly evolved terrestrial arthropods transfer semen by true copulation, the spermatophore is retained in many other terrestrial arthropods. Many crustaceans, though primarily aquatic, retain spermatophores. The spermatophores of decapod Crustacea can be grouped as pedunculate and non-pedunculate types (see Calman, 1909). In the former, external fertilization is typical (*e.g.,* anomurans), whereas in the brachyuran crabs internal fertilization occurs. Contrary to Brachyura and Macrura, among anomurans the spermatophores show great morphological diversity in the number of spermatophores in a ribbon, shape of the ampule, and the length of peduncle (Mouchet, 1931). In the macrurans, the spermatophore is a non-pedunculate, tubular mass embedded in a mucoid matrix that aids attachment to the female body.

Crustacean spermatophores occur in many forms, but little is known of their chemical composition. Previous authors have attributed the stabilization of spermatophore walls to chitin (Spalding, 1942; King, 1948). Recently, Malek and Bawab (1971) reported phenolic tanning in the spermatophore of the marine prawn *Penaeus*

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Abbreviations: PVD, proximal vas deferens; DVD, distal vas deferens; VD, vas deferens; AMP, acid mucopolysaccharide.

trisulcatus similar to cuticular tanning; Uma and Subramoniam (1979) did not find such hardening in the brachyuran crab *Scylla serrata*. Interestingly, in the spermatophores epizoically deposited on the female marine crustaceans, the membranous sperm sacs are invariably embedded in a thick gelatinous matrix (Berry and Heydorn, 1970). However, relatively little is known about the physiological role, if any, of these mucoid substances in sperm transport. Functional significance of such copious secretions of mucus warrants knowledge of their chemical composition.

Mole crabs of the family Hippidae deposit a macruran type of spermatophoric mass (Matthews, 1956; Subramoniam, 1977a). In the present study, two sand crabs *Emerita asiatica* and *Albunea symnista* were selected for studying the origin, chemical composition, and the strategic role of spermatophoric mass in sperm transfer.

Materials and Methods

Emerita asiatica and *Albunea symnista* are intertidal forms found on the sandy beaches of Madras coast, India.

The testes and vas deferens in *E. asiatica* were removed by pulling the fifth leg at its base with forceps (Subramoniam, 1977a). The male reproductive system of *A. symnista* was dissected out for histological studies. The arrangement and structure of the unfixed spermatophoric mass in the two crabs were observed with bright field and phase contrast optics after vital staining. Paraffin sections of Bouins or formaldehyde fixed vas deferens were stained in Heidenhain's hematoxylin and eosin.

Histochemical properties of the spermatophoric mass were studied in paraffin and cryostat sections of vas deferens. Histochemical procedures were mainly from Pearse (1968) and Chayan *et al.* (1973). Fresh spermatophores removed from the distal vas deferens were also used. Spermatophores deposited on the female crabs were not used for histochemistry because of possible salt contamination from sea water. Mineral salts can interfere with the reactivity of complex carbohydrates (Aminoff *et al.*, 1970). Histochemical identification and characterization of the mucopolysaccharides were made by 1) periodate oxidation of *vic*-glycols, 2) detection of acid groups with basic dyes, and 3) combination of basic dye and *vic*-glycol methods (Alcian blue-PAS) for differentiating the acid mucopolysaccharides from neutral mucopolysaccharides (Spicer, 1960). Differences in the acidic characteristics of mucopolysaccharide were observed by using the basic dyes at controlled pH and at different critical electrolyte concentrations. In addition, blocking of staining by specific reactions such as methylation and acetylation were also carried out. Phenolic substances in the spermatophoric mass were located in the fresh unfixed as well as paraffin sections using ferric chloride, Millon's test, and catechol incubation techniques (Johri and Smyth, 1956; Pearse, 1968). Other histochemical tests are given at appropriate places in the text and tables.

Results

Albunea symnista

Reproductive system

The male reproductive system consists of paired testes in the cephalothoracic region, and its posterior part continuing as the vas deferens (VD). This runs posteriorly for a short distance and then turns to the anterior side, forming the descending and ascending limbs (Fig. 1a). The ascending limb again turns back to open at the base of the fifth walking leg. The entire VD is divisible into a short proximal and a dilated distal portion (Fig. 1a).

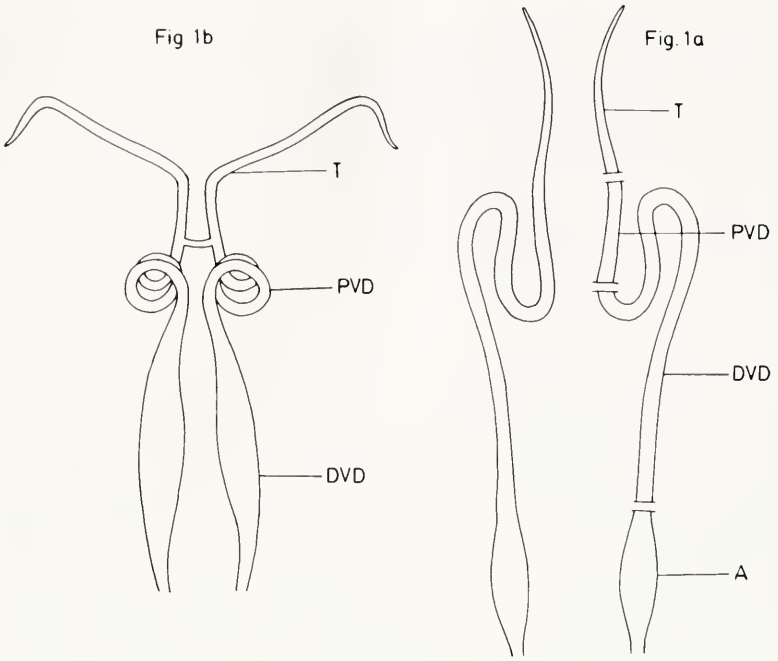


FIGURE 1. a. Diagrammatic representation of the male reproductive system of *A. symnista*. b. Diagrammatic representation of the male reproductive system of *E. asiatica*. T = testis; PVD = proximal vas deferens; DVD = distal vas deferens; A = ampulla.

Arrangement of spermatophore inside VD

The mature vesiculate spermatozoa, as released from the testes, are clumped in the anterior-most part of the proximal VD (Figs. 2, 3). This sperm mass is ensheathed in a thin wall formed by the condensation of secretory material probably emanating from the hyperactive epithelial cells (Fig. 4). This walled sperm mass passes into the enlarged distal portion of the VD. The completed spermatophore is a straight tube without accessory secretion adhering to it. As the tubular spermatophore enters the first part of the distal vas deferens (DVD), it twists and lies apposed to the ventral epithelial wall. This twisted tube, as it extends distally, becomes folded into loops and is set on to a firm membrane, forming a continuous cord in the ventral region (Figs. 5, 8). Between the loops the spermatophoric tube tends to constrict towards the ventral region and often forms node-like structures, apparently interrupting the continuity of the spermatophoric tube (Figs. 6, 7). This arrangement is regular and is found even in the extruded spermatophoric mass.

In the DVD the ventral gelatinous cord is thickened and connects the highly convoluted spermatophoric ribbon ventrally. The epithelial cells in the dorsal region of the DVD produce two typhlosole-like structures that secrete yet another material into the lumen (Fig. 9). This secretion fills up the rest of the lumen containing the spermatophoric ribbon and mixes with the secretions of the ventral epithelial cells, namely the gelatinous cord.

Histology of VD

The proximal portion is highly secretory, as evidenced by a very thick inner epithelial layer and a very thin outer connective tissue layer. The lumen is small and

circular (Fig. 2). The thickness of the inner epithelial cells gradually becomes unequal; in the ventral region they are thin; in the dorsal portion they are long columnar cells (Fig. 3). The mode of secretion of these glandular cells seems to be holocrine, as evidenced by the rupture of cell membrane to release the cytoplasmic contents (Fig. 4). The hyperactive nuclei are arranged basally and the cell membranes are not distinct (Fig. 4). The epithelial cells and their secretion stain a dark blue color with hematoxylin and eosin. The released secretory material condenses into a thin membrane, which encloses the sperm mass. The dorsal glandular epithelial cells of the dilated DVD are transformed into two typhlosole-like structures whereas in the ventral region the epithelial cells are thin but highly secretory in nature (Fig. 9). The thickness of the VD wall from this region onwards results mainly from thickened multilayered outer circular and inner longitudinal muscle fibers, which probably forcibly push viscous spermatophore out. There is a bundle of connective tissue in the interior of the two typhlosoles over which there are secretory epithelial cells, producing a conical shape (Fig. 10).

The secretory material originating from the ventral epithelial cells is granular, but later coalesces to form a continuous gelatinous cord (Fig. 11). The typhlosoles and adjoining dorsal epithelial cells produce a rather less viscous fluid that is deposited in a lamellar fashion (Fig. 12). Minute granules are dispersed in this matrix. The epithelial cells are gradually reduced in thickness and the typhlosoles are also reduced in size towards the posterior part of VD (Fig. 12). In the ampullar region, the spermatophore is highly folded and the protective gelatinous matrix is very viscous (Figs. 13, 14). The spermatophore wall is very prominent and refractile, and the mature spermatozoa contained within it are vesicular in shape (Fig. 14).

Chemical composition of spermatophoric mass

The fully-formed spermatophoric mass of *A. symnista* consists of three components: the tubular spermatophore, a gelatinous cord, and the protective gelatinous matrix. Results of the histochemical analysis are given in Tables I and II. Details are given below.

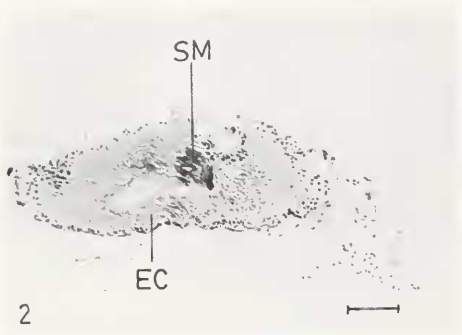
Spermatophore

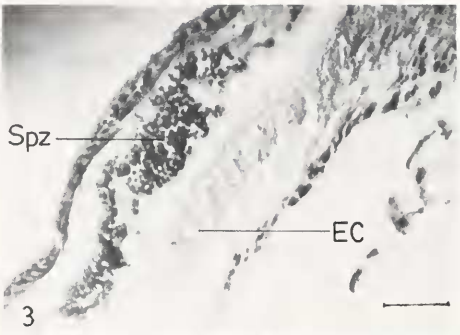
The spermatophore wall is mainly composed of a neutral mucopolysaccharide, as evidenced by the PAS positivity in Alcian blue (AB) and PAS combination. With Best's carmine, this layer is a weak red color which is not abolished by diastase treatment, indicating that it is mainly mucoid in nature. Interestingly, this neutral mucopolysaccharide does not include chitin, as shown by the negative chitosan test.

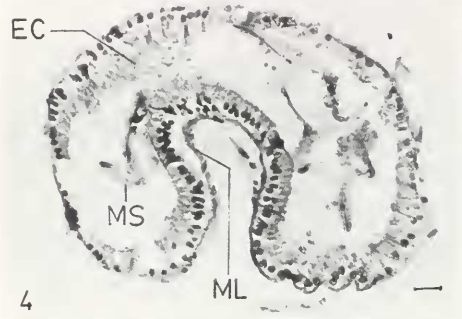
The protein part of this mucopolysaccharide includes basic groups as well as tryptophanyl groups. Disulphide and sulphydryl groups are absent. Reactions to lipid tests are negative (Table I).

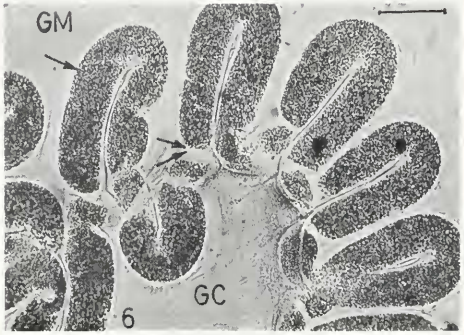
Sperm mass

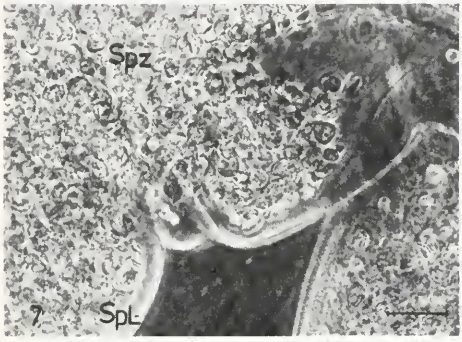
The binding substance of the spermatozoa stains metachromaticly with toluidine blue, suggesting strongly acidic groups in the mucus. These acidic groups are in the form of sulfate polyanions, as revealed by the specific benzidine reaction of Bracco-Curti's method and the intense staining reaction with aldehyde fuchsin. However, such substances are lacking inside the spermatozoa which gave a diastase labile PAS positive reaction in the axial structure of the acrosomal vesicle. Such a differential histochemical property between the sperm mass substance and the spermatozoa is

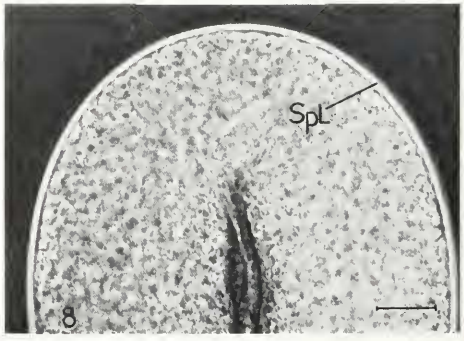


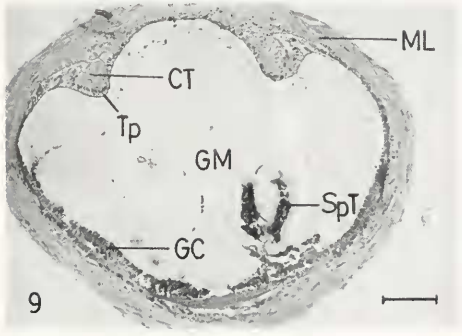


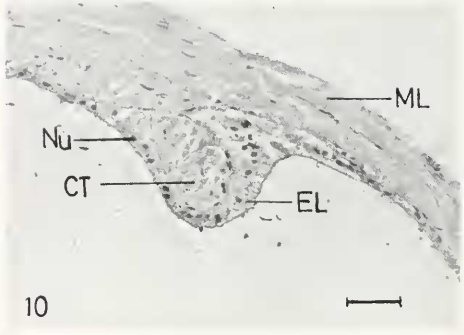












FIGURES 2–10. *Albunea symnista.*

FIGURE 2. Transverse section through the anterior most part of the PVD showing the thickened epithelial cells. SM = Sperm mass substance; EC = Epithelial cells. Bar = 155 μm.

Fig. 5

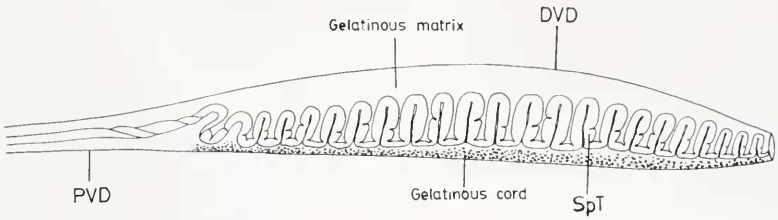


FIGURE 5. Diagrammatic representation of the part of the PVD and DVD showing the origin and arrangement of the spermatophoric mass, consisting of a convoluted spermatophoric tube (SpT), gelatinous matrix, and the ventral gelatinous cord. Note the constrictions in the spermatophoric tube in the DVD region.

clearly observed in the AB-PAS technique and with aldehyde fuchsin. Among proteins, acidic, —SH, and tryptophanyl groups are present.

Gelatinous cord

In Mallory's triple stain, the precursor granules and gelatinous cord stain red; in hematoxylin they stain blue. The gelatinous cord contains a partly diastase-digestible PAS positive substance conjugated to a protein, rich in basic and aromatic groups. A positivity to Best's carmine may indicate glycogen, but after diastase digestion the gelatinous cord continues to give a positive reaction, suggesting a mucin. Appearance of magenta with the AB-PAS technique and weak staining reaction with aldehyde fuchsin suggest neutral mucopolysaccharides. They give a positive reaction to catechol incubation indicating the enzyme phenolase. When incubated in catechol, the entire connecting cord became brown. Lipid has also been indicated by the positive Sudan black B test. Similar histochemical results were obtained for the precursor granules of the gelatinous cord.

FIGURE 3. Longitudinal section of the anterior part of the PVD showing the agglutination of spermatozoa (Spz) into a long cord. EC = Epithelial cells. Bar = 200 μm.

FIGURE 4. Transverse section of the PVD at the convoluted region showing the nature of the secretory epithelial cells (EC) surrounded by a thin muscular layer (ML); the secretory materials are found in as membranous substances (MS). Bar = 50 μm.

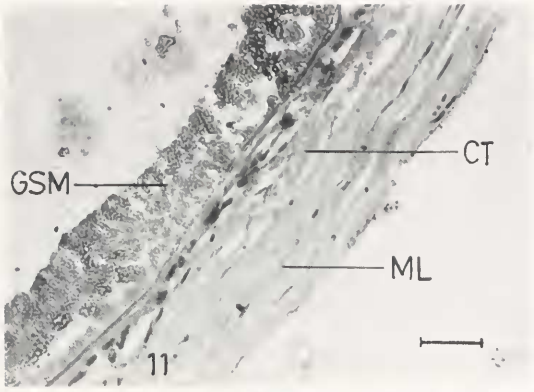
FIGURE 6. Freshly extended spermatophoric mass showing the loop-like convolution of the spermatophoric tube (↑), joined ventrally by the connecting gelatinous cord (GC) and embedded in the gelatinous matrix (GM). Note the discontinuity of the inner spermatophoric tube (↑↑) by the node formation. Bar = 10 μm.

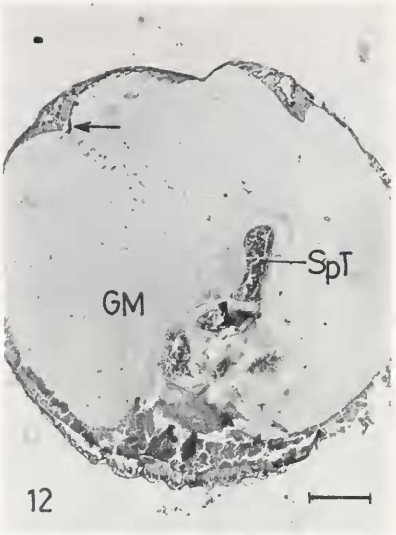
FIGURE 7. Higher magnification of spermatophoric tube at the region of node formation under phase contrast. Spz = spermatozoa; SpL = spermatophore layer. Bar = 50 μm.

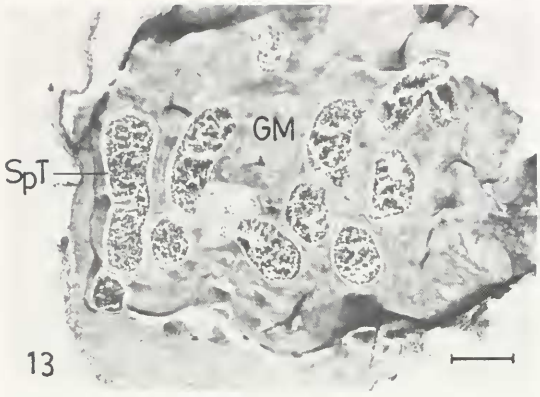
FIGURE 8. Higher magnification of the spermatophoric tube at the region of convolution under phase contrast showing the refractile nature of the spermatophore layer (SpL). Bar = 50 μm.

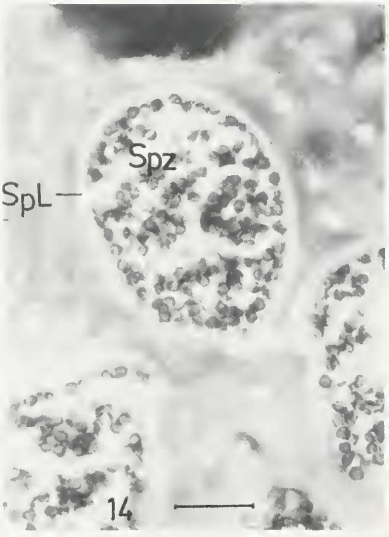
FIGURE 9. Transverse section of the DVD showing the two dorso-laterally placed typhlosoles (Tp), thick connective tissue (CT), and muscular layer (ML); the lumen is filled with gelatinous matrix (GM). Note the spermatophoric tube (SpT) at the ventral region, embedded on the gelatinous cord (GC). Bar = 155 μm.

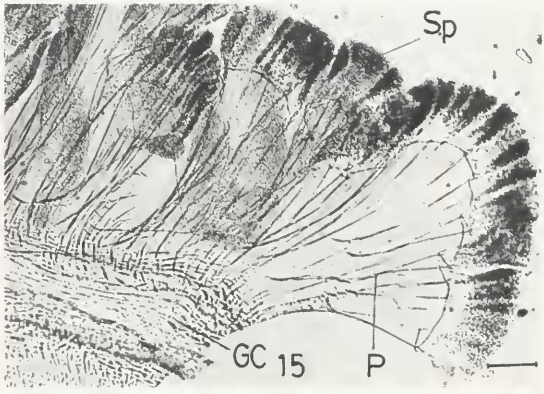
FIGURE 10. Higher magnification of the typhlosole. Note the central connective tissue (CT) lined by the secretory epithelial layer (EL). Nu = nucleus; ML = Muscular layer. Bar = 60 μm.

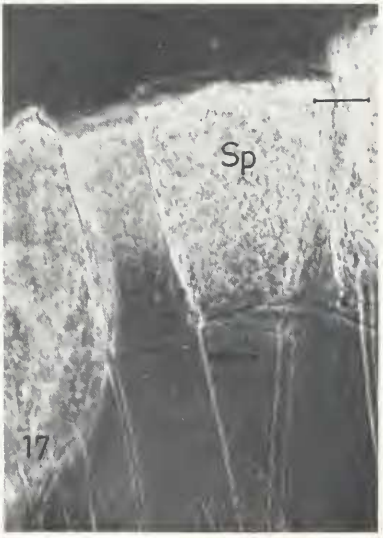

GSM
CT
ML
11


SpT
GM
12


SpT
GM
13


SpL
Spz
14


Sp
GC
15
P


Sp
17


SpL
16

Gelatinous matrix

The gelatinous matrix becomes blue with Mallory's triple stain and orange red with hematoxylin-eosin combination. It is metachromatic to the basic dye, toluidine blue. However, the staining intensity is greater with alcian blue than with toluidine blue (Table I). The acidic nature of mucopolysaccharide substances of the gelatinous matrix is also revealed from the AB-PAS combination. However, mild methylation did not remove basophilia obtained in the gelatinous matrix. The absence of sulphated groups is indicated by a negative reaction to Bracco-Curti's. Aldehyde fuchsin stain for differentiation of carboxyl and sulphated groups (Pearse, 1968) gives only a weak reaction. With toluidine blue the metachromatic reactions are obtained in high pH suggesting that the carboxyl groups may be predominant (Table I). However, the staining intensity with alcian blue at critical electrolyte concentration did not reveal much difference. In all the histochemical tests using alcian blue, a very strong reaction has been obtained with the gelatinous matrix.

In tests for protein, mercuric bromophenol blue gave a positive reaction. Tests for basic proteins were negative. Tests with performic acid alcian blue were positive. However this is not due to disulphide groups because the positive reaction occurred even after thioglycollate treatment. This is confirmed by the negative result of the PFAS test. Gelatinous matrix is not positive to tests for catechol, tyrosine, and other phenol containing substances, although tryptophanyl groups were detected by the DMAB test (Table II). Lipids are absent.

Emerita asiatica
Reproductive system

The vasa deferentia originate where the two limbs of the testes fuse (Fig. 1b). The VD in the proximal region is thin, convoluted, and then runs posteriorly as a straight tube gradually increasing in size.

Arrangement of spermatophores

The deposited spermatophores consist of two types of pedunculate spermatophores, one of truncated cone shape, the other of tumbler shape (Figs. 15, 16, 17). The

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FIGURES 11–14. *Albunea symnista.*

FIGURE 11. Higher magnification of the ventral wall of the DVD showing the outer thick muscular layer (ML), inner connective tissue (CT), and the concentration of granular secretory materials (GSM) forming the ventral gelatinous cord. Bar = 40 μm.

FIGURE 12. Transverse section through the distal most part of DVD showing the lamellated nature of the gelatinous matrix (GM) interspersed by granular depositions. Note the shrinking of the typhlosoles (↑) and the adjoining epithelial cells. SpT = spermatophoric tube—stained in AB-PAS. Bar = 155 μm.

FIGURE 13. Transverse section through the ampullar region showing the highly folded spermatophoric tube (spT), embedded in viscous gelatinous matrix (GM). Bar = 50 μm.

FIGURE 14. Higher magnification of the spermatophoric tube, its layer (SpL), and the vesicular spermatozoa (Spz). Bar = 40 μm.

FIGURES 15–22. *Emerita asiatica.*

FIGURE 15. Spermatophoric ribbon of *E. asiatica* showing the arrangement of the spermatophores (Sp) in the form of a ribbon with the peduncle (P) connected to the connecting gelatinous cords (GC) which, in freshly extruded condition, is fibrous. Bar = 200 μm.

FIGURE 16. Truncated shaped spermatophores of *E. asiatica.* Note the crystalline nature of the spermatophore layer (SpL) and the nipple-like projection (↑) through which the dehiscence of spermatozoa occurs. Note the drawn out peduncle (↑↑). Phase contrast. Bar = 100 μm.

FIGURE 17. Tumbler-shaped spermatophores (Sp). Note the two drawn out peduncles (↑). Phase contrast. Bar = 100 μm.

TABLE I

Histochemical characteristics of mucopolysaccharide substances and lipids of spermatophoric mass of Albunea symnista

Tests	Sperm mass*	Spermatophore wall	Gelatinous cord	Gelatinous matrix
Best's carmine (BC)	++ R	+ R	+++ R	+ R
Diastase + BC	+ R	+ R	++ R	±
Schiff alone	–	–	–	–
Periodic acid Schiff (PAS)	+ M Sperm cells	++ M	+++ M	±
Diastase + PAS	– Sperm cells	±	++	±
Acetylation + PAS	–	+ M	+ M	–
Delipidation + PAS	±	+ M	++ M	+ M
Alcian blue – PAS (AB – PAS)	++ M Sperm cells + B sperm mass substance	+++ M	+++ M	+++ B
AB – PAS after mild methylation	++ M Sperm cells + B sperm mass substance	+++ M	+++ M	+++ B
AB – PAS after strong methylation	++ M Sperm cells + M sperm mass substance	+++ M	+++ M	+++ B
Aldehyde fuchsin	+++ P	–	±	+ P
Bracco-Curti	++ BB	–	–	–
Toluidine blue at different pH				
pH 1	+++ V	–	–	+ V
pH 3	+++ V	–	–	++ V
pH 4	++ BV	–	–	++ V
pH 7	++ B	–	+ B	+++ BV

TABLE I (*Continued*)

Tests	Sperm mass*	Spermatophore wall	Gelatinous cord	Gelatinous matrix
Alcian blue: critical electrolyte concentrations of $MgCl_2$				
0.2 *M*	+ B	–	–	++ B
0.6 *M*	±	–	–	++ B
0.8 *M*	+ B	–	–	++ B
1.0 *M*	++ B	–	–	+ B
1% Aqueous alcian blue	+ B	–	±	+ B
Chitosan	–	–	–	–
LIPID				
Sudan black B (SBB)	–	–	±	±
Delipidation + SBB	–	–	–	–
1% Nile blue	+++ B	–	–	++ B

B = Blue; BB = Benzidine Blue; BV = Bluish violet; M = Magenta; P = Pink; R = Red; V = Violet; – = negative; ± = doubtful; + = moderately positive; ++ = positive; +++ = intensely positive; * = sperm mass refers collectively to sperm mass substance, that binds the sperm cells within the spermatophore as well as the sperm cells. When reactions are distinct for sperm cells and sperm mass substance they are indicated accordingly.

peduncles are connected ventrally to filaments, which run along the entire length of the spermatophoric ribbon (Fig. 15). The whole structure is embedded in a gelatinous matrix which binds the spermatophores tightly.

Histology of VD

The spermatophores originate in the anterior region of VD. Here, the rod-shaped mature spermatozoa are agglutinated into many clusters (Fig. 18). Each cluster of sperm is covered by a gelatinous membrane probably originating from the secretory inner columnar epithelial cells (Fig. 18). The epithelial cells are covered by a thin layer of connective tissues. A muscular layer is lacking. The epithelial layer gradually increases in thickness in the lateral sides with mid ventral and dorsal portions being atrophied into a thin layer (Fig. 19). Evidence for the formation of the peduncle from the ventral epithelium are seen in this region (Fig. 19).

In DVD, the inner epithelial cells undergo morphological changes including a shortening of the cells. In the dorsal region, they produce a typhlosole-like projection (Fig. 20). However, this differs from the typhlosoles of *A. symnista* by the absence of the inpushing of the inner connective tissues. The typhlosole secretes a frothy substance which fills the entire lumen of DVD. The ventrolateral epithelial cells secrete a gelatinous material which is deposited on the surface of epithelial cells in a manner akin to the lamellated endocuticle.

This gelatinous layer on the ventral luminal surface of the VD further condenses to form a thick cord. This branches linking itself to every one of the spermatophore

TABLE II

Histochemical characteristics of proteinaceous substances of spermatophoric mass of Albunea symnista

Tests	Sperm mass	Spermatophore wall	Gelatinous cord	Gelatinous matrix
Mercuric bromophenol blue	+ B	+ B	+ B	+ B
0.1% Aqueous bromophenol blue (ABB)	+ B	+ B	+++ B	±
Deamination + ABB	–	–	–	–
0.1% Aqueous toluidine blue (TB)	+++ V	–	–	++ V
Methylation + TB	–	–	–	–
Methylation + Demethylation + TB	+ V	–	–	+ V
Dimethylaminobenzaldehyde (DMAB)	+ B	+ B	++ B	++ B
40% Formaldehyde + DMAB	–	–	–	–
Millon's	–	–	+ BR	+ BR
Bromination + Millon's	–	–	–	–
Performic acid alcian blue (PFAB)	–	–	–	+ B
Thioglycollate + PFAB	–	–	–	+ B
Performic acid Schiff	–	–	–	–
Ferric ferricyanide (FFC)	++ PB	–	–	–
Mercuric chloride + FFC	–	–	–	–
Ferric chloride	–	–	–	–
Catechol incubation	–	–	+ Br	–
Catechol + Pretreatment with formalin	–	–	++ Br	–
Catechol + Pretreatment with alcohol	–	–	+ Br	–
TB + Light green (Anderson and Weis-Fogh, 1964)	– G	–	–	–
Methylene blue in glycerol (Anderson and Weis-Fogh, 1964)	–	–	–	–

B = Blue; BR = Brick Red; Br = Brown; G = Green; PB = Prussian Blue; V = Violet.
– = negative; ± = doubtful; + = moderately positive; ++ = positive; +++ = intensely positive.

ampules, which lie distributed on the dorso-lateral periphery of the lumen, providing, besides, an outer covering for them (Figs. 21, 22). In the distal region, the epithelial cells including typhlosole are greatly reduced.

The histochemistry of spermatophoric components

Histochemically the spermatophoric mass in *E. asiatica* is similar to *A. symnista* (Table III, IV). The sperm mass substance is composed of highly sulphated acidic mucopolysaccharides, whereas the inner layer of the spermatophore contains carboxylated AMP. In contrast, the ventral gelatinous cord, peduncle, and the outer layer of spermatophore ampullae when inside the VD, are PAS-positive. The entire gelatinous matrix stains blue in AB-PAS indicating its acidic nature. The gelatinous matrix also contains vicinyl hydroxyl groups as revealed by a PAS positivity, when used alone. Further, the acidic group of AMP is carboxylic in nature. The outer layer of the spermatophore in the freshly extruded condition is refractile to all stains. The spermatophoric mass of *E. asiatica* does not undergo 'hardening' on exposure to sea water.

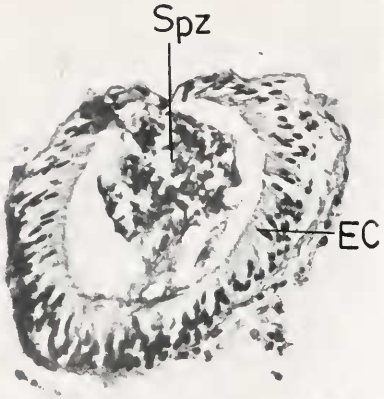
The basal gelatinous layer is highly eosinophilic, but the stalk arising from it stains blue with hematoxylin. This change in the staining reaction of the gelatinous layer towards the formation of the peduncles and possibly the outer layer of the spermatophore suggests a corresponding transformation in the chemical composition, especially proteins. The gelatinous layer and the peduncle stain intensely with Millon's reagent especially in fresh material suggesting the presence of tyrosine (Table IV). Dihydroxyphenols are not detected in any of the spermatophoric components using the ferric chloride test. However, strong phenolase activity is observed in the ventral gelatinous cord (Table IV). The crystalline spermatophore layer and the peduncle do not give a positive reaction to this test.

Discussion

Pedunculate spermatophores are characteristic of Anomura. However, a macruran type of spermatophoric mass has been reported for a few members of the family Hippidae (Matthews, 1956; Subramoniam, 1977a). The present study suggests interesting possibilities in the evolution of pedunculate spermatophores from the non-pedunculate spermatophoric ribbon.

In both *A. symnista* and *E. asiatica* the spermatophore proper is supported by a basal gelatinous cord and an impregnating gelatinous matrix (Table V). This resembles several of the panulirid spiny lobster spermatophores, described by Berry and Heydorn (1970). In *A. symnista* the spermatophore is tubular with apparent node-like constrictions leading to internal discontinuities; in *E. asiatica* it is broken up into ampullae with drawn out peduncles. Interestingly, in two hermit crabs, *Dardanus asper* and *Pagurus novae zealandiae,* Matthews (1953) and Greenwood (1972) respectively, found evidence that the segmentation of the continuous sperm sheath leading to stalked spermatophore results from specialized muscular activity and modified lumen shape of the VD. The breaking up of the continuous spermatophoric tube by constrictions (*Albunea*) and distinct spermatophoric ampullae with drawn out peduncles set on a basal filamentous pedestal (*Emerita*) suggests that these anomuran sand crabs may be midway forms in the evolution of discrete pedunculate spermatophores of other anomurans from the tubular spermatophores of Macrura.

In the brachyuran decapods, the PVD is secretory and DVD has a storage function (Hinsch and Walker, 1974). However, the macruran and anomuran DVD too is highly secretory producing the accessory mucoid spermatophoric substances (Matthews, 1951; Berry and Heydorn, 1970). Furthermore, to increase the surface area of the glandular epithelial cells the DVD possesses typhlosole-like inpushings. In *E. asiatica,* the typhlosole corresponds to that of the spiny lobster, *Panulirus homarus* (Berry and Heydorn, 1970) in that the typhlosole consists of long columnar cells with mul-



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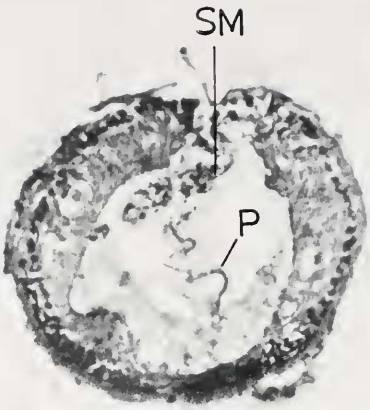

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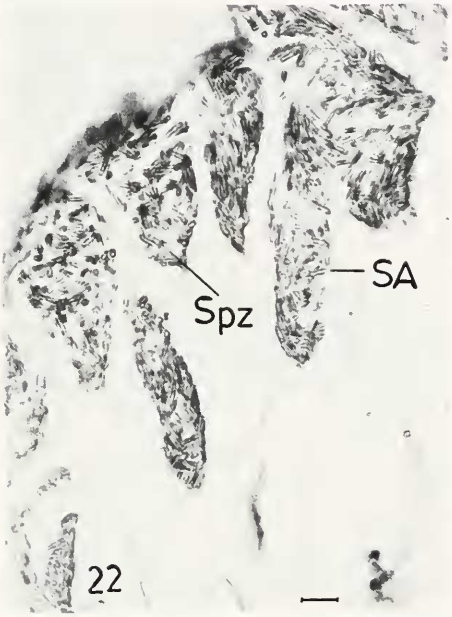

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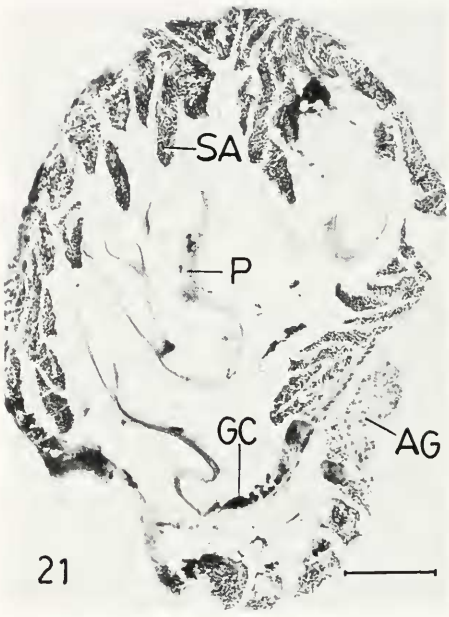

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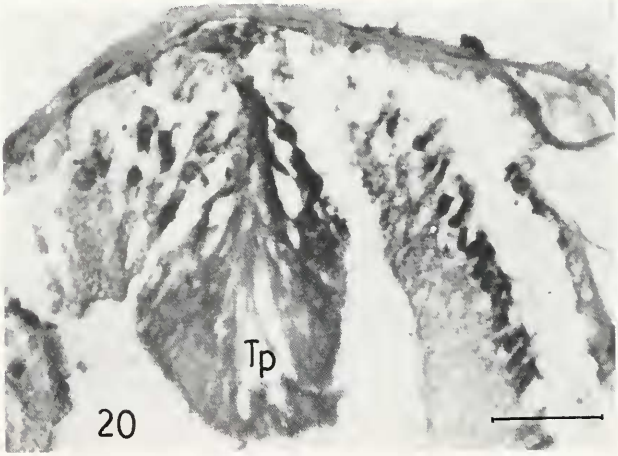

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tilobation at the peripheral region forming the shape of a leaf. However, in *A. symnista* there are two conical shaped dorsolaterally placed typhlosoles.

Mucopolysaccharides form the main components of the spermatophores of *A. symnista* and *E. asiatica*. AB-PAS differentiates the acidic mucopolysaccharides of the sperm mass-substance and the gelatinous matrix from the neutral mucopolysaccharides of the ventral gelatinous cord (Table V). Toluidine blue staining at different pHs indicates that the acidic mucopolysaccharides of the sperm mass substance and gelatinous matrix of *A. symnista* are rich in sulphated and carboxylated groups, respectively. Spicer (1960) suggests that mild methylation hydrolyses the sulphated groups and esterifies the carboxylated groups. With this treatment before the AB-PAS test, neither the $-COOH$ groups of gelatinous matrix nor the $-SO_4$ groups in the mucopolysaccharides of sperm mass substance could be removed. However, prolonged methylation resulted in the removal of basophilia in sulphated acid-mucopolysaccharides and the assumption of PAS positivity suggesting the complete hydrolyses of $-SO_4$ groups. At the same time, the gelatinous matrix did not lose basophilia suggesting that the carboxylated groups may be enormous. These acidic groups may bind inorganic ions such as calcium ions (Aminoff *et al.*, 1970) from sea water to produce 'hardening' of the spermatophore into a putty-like mass.

The gelatinous matrix of *E. asiatica* contains periodate reactive acid mucopolysaccharides. Such a mucopolysaccharide heterogeneity has also been reported in the epithelial mucin of several vertebrate systems (Spicer and Duvenei, 1964) and in the vas deferens epithelial mucin of a pulmonate slug (Els, 1974). The predominance of various muco-substances in the spermatophoric components of the two sand crabs (see Table V) may be correlated to their protective as well as structural functions (Jeanloz, 1970; Montgomery, 1970). Though carbohydrate forms the major component of mucopolysaccharide substances, only meager amounts of glycogen are found suggesting that the spermatophoric components may not have a nutritive function. Interestingly, the sperm cells contain a significant quantity of glycogen, possibly for endogenous energy metabolism.

A similarity of the crustacean spermatophore to arthropod cuticle has been proposed because of the possible occurrence of phenolic tanning in the spermatophore wall (Malek and Bawab, 1971) and a chitin-protein like lamellar pattern (Gharagozlov-van Ginneken, 1978). In the spermatophores of *E. asiatica* (and to a lesser extent *A. symnista*), there is evidence for tyrosine-rich protein and the enzyme phenolase. However, no diphenols have been detected. Possibly, the tyrosyl residues, in the presence of phenolase might be oxidised *in situ* to quinones which tan the protein. This mechanism, called 'self-tanning' (Malek and Bawab, 1971; Hackman, 1974) has

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FIGURE 18. Transverse section through the anterior part of the PVD showing the columnar nature of the inner secretory epithelial cells (EC) and the agglutination of the spermatozoa (Spz) in the lumen. Bar = 20 μm.

FIGURE 19. Transverse section through another part of the PVD showing the sperm mass (SM) as well as the formation of the peduncle (P). Note the change in the shape of the lumen. Bar = 20 μm.

FIGURE 20. Transverse section through the proximal part of the DVD showing the leaf-like typhlosole (Tp). Note the absence of connective tissue inside the typhlosole. EC—epithelial cells. Bar = 50 μm.

FIGURE 21. Transverse section through the distal part of DVD showing the final arrangement of the spermatophoric ribbon with the spermatophoric ampule (SA) arranged on the dorso-lateral region. Each spermatophoric ampule is connected by the peduncle (P), originating from the ventral connecting cord (GC). AG—androgenic gland. Bar = 100 μm.

FIGURE 22. A closer view of the transverse section through spermatophoric ampule (SA) showing the haphazard arrangement of the rod-shaped spermatozoa (Spz). Bar = 10 μm.

TABLE III

Histochemical characteristics of mucopolysaccharide substances of spermatophoric mass of Emerita asiatica

Tests	Sperm mass*	Spermatophore Inner/Outer layer	Peduncle/ gelatinous cord	Gelatinous matrix
Best's carmine	+ R	+/+ R/R	++/++ R/R	+ R
Schiff alone	−	−/−	−/−	−
Periodic acid Schiff (PAS)	+++ M Sperm cells	+++/+ M/M	++/++ M/M	+ M
Alcian blue − PAS	++ M Sperm cells + B sperm mass substance	++/+ M/M	+/+ M/M	+++ B
Aldehyde fuchsin	++ B	+/− P	−/−	+ P
Bracco-Curti	++ BB	−/−	−/−	−
Toluidine blue at different pH				
pH 1	++ V	+/− B	±/±	+ V
pH 3	++ V	++/− B	+/+ V/V	++ V
pH 4	++ BV	+++/− B	+/+ V/V	+++ V
pH 7	++ B	+++/− V	+/+ B/B	+ V
Alcian blue: critical electrolyte concentrations of $MgCl_2$				
0.2 *M*	+ B	+/± B	+/+ B/B	++ B
0.6 *M*	+ B	+/− B	−/−	++ B
0.8 *M*	±	±/±	−/−	++ B
1.0 *M*	+ B	−/−	−/−	++ B
1% Aqueous alcian blue	+ B	+/± B	±/±	++ B
Chitosan	−	−/−	−/−	−

B = Blue; BB = Benzidine Blue; Br = Brown; BV = Bluish Violet; M = Magenta; P = Pink; R = Red; V = Violet; * = Sperm mass refers collectively to sperm mass substance, that binds the sperm cells within the spermatophore as well as the sperm cells. When reactions are distinct for sperm cells and sperm mass substance they are indicated accordingly.

TABLE IV

Histochemical characteristics of proteinaceous substances of spermatophoric mass of Emerita asiatica

Tests	Sperm mass	Spermatophore Inner/Outer layer	Peduncle/ gelatinous cord	Gelatinous matrix
0.1% Aqueous bromophenol blue	++ B	++ B / + B	+ B / B	+ B
0.1% Aqueous toluidine blue (TB)	++ V	++ V / ++ V	++ V / ++ V	++ V
Dimethylaminobenzaldehyde (DMAB)	+ B	+ B / + B	+ B / B	+ B
Millon's	−	−/−	+ BR / + BR	+ BR
Performic acid alcian blue (PFAB)	−	−/−	−/−	++ B
Performic acid Schiff	+ P	+ P / −	−/−	−
Ferric ferricyanide	+	+ PB / −	−/−	−
Ferric chloride	−	−/−	−/−	−
Catechol incubation	−	−/−	+ Br / + Br	−
Catechol + Pretreatment with formalin	−	−/−	++ Br / ++ Br	−
Catechol + Pretreatment with alcohol	−	−/−	−/−	−
LIPID				
Sudan Black B	−	−/−	−/−	+ Bl.B
1% Nile blue	+ B	+ B / −	−/−	−

B = Blue; BR = Brick Red; Br = Brown; P = Pink; V = Violet; Bl.B = Bluish black.
− = negative; ± = doubtful; + = moderately positive; ++ = positive; +++ = intensely positive.

been reported for the adhesive cement of the cirripede, *Balanus crenatus* (Linder and Dooley, 1973). More importantly, the phenolic compounds may have other roles such as antimicrobial activity (Brunet, 1980) for the exposed spermatophores.

The structural difference in the spermatophores of *E. asiatica* and *A. symnista* may also be correlated with the mechanism of dehiscence. In *E. asiatica* the spermatophore deposition always occurs before egg extrusion and no dehiscence takes place until the egg mass comes in contact with the spermatophores (Subramoniam, 1977a; his Figs. 1G–1J). He also suggested that an oviductal secretion is responsible for the digestion of the cementing material closing the lip of the spermatophore. However, in *Albunea* this mechanism may not apply because the thick protective

TABLE V

Summary of the results concerning the characterization of mucopolysaccharides of the spermatophoric components of A. symnista *and* E. asiatica

Spermatophoric components	Chemical nature	Origin
Albunea symnista		
1. Sperm mass substance	Sulphated AMP	PVD
2. Spermatophore wall	Neutral MP	PVD
3. Gelatinous cord	Neutral MP	DVD, Ventral epithelium
4. Gelatinous matrix	Carboxylated AMP	DVD, Dorsal epithelium especially typhlosole.
Emerita asiatica		
1. Sperm mass substance	Sulphated AMP	PVD
2. Spermatophore wall	Carboxylated AMP	PVD
Inner layer		
Outer layer	Neutral MP	DVD, Ventral epithelium
3. Peduncle/gelatinous cord	Neutral MP	DVD, Ventral epithelium
4. Gelatinous matrix	Periodate reactive AMP	DVD, Dorsal epithelium especially typhlosole

matrix hardens into a putty-like substance on exposure to sea water. It is possible that the females use their powerful chelae to remove the protective matrix and then gouge the spermatophoric tube open to release the spermatozoa at the time of fertilization, as reported in the spiny lobsters (Fielder, 1964; Berry, 1969).

The two sand crabs, *E. asiatica* and *A. symnista* inhabit the shifting sands of the surf zone. Hence, the sticky spermatophoric ribbon in these crabs has great adaptive value because it can be deposited quickly and firmly. Further, in *E. asiatica,* spawning rapidly follows spermatophore deposition (Subramoniam, 1977b) and hence the spermatophoric ribbon remains as a jelly. Conversely, there seems to be a long time period between mating and spawning in *A. symnista* (unpub. obs.) with a necessity for the spermatophore to undergo 'hardening.' These factors may contribute to the reproductive success of the sand crabs, especially *E. asiatica* (Subramoniam, 1977b, 1979, 1981).

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